



Effects of Diet on Inflammatory Responses in the Intestines

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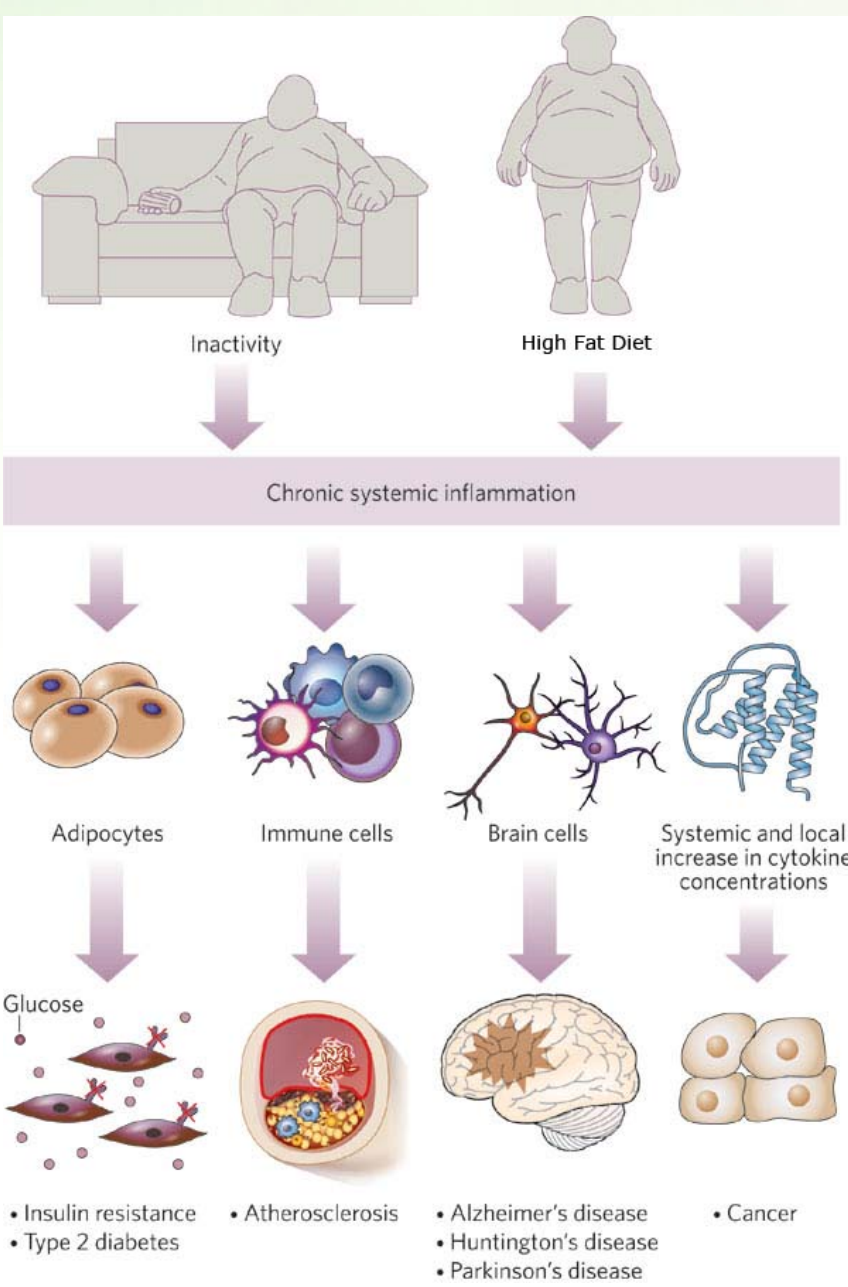
ABSTRACT

During our time in the Olefsky Laboratory, we have been analyzing the immune system's response to a change in diet, and we have been doing so specifically in the distal small intestine and the colon. In our experiments to investigate the subject, we examined the intestines of mice on differing diets to look for signs of inflammation, such as macrophages and eosinophils, which are two of the most contributing factors to a normal immune response. Our results have concluded that there are fewer macrophages and eosinophils in the intestines of mice on high-fat diets, therefore showing that there are reduced activations of the immune system in the intestines of those mice.

Background Information and General Procedure

Over the past few years there have been major changes in the way scientists view many diseases. There has been a shift to a focus of the role of chronic inflammation and its effects on those significant worldwide diseases. Inflammation is generally beneficial for the body because it brings the immune system's attention to the area of the body at risk of infection from any pathogen. In some cases however, the inflammation does not stop, and this may be caused by changes in the normal bacteria that are usually in the body.

The 100 trillion bacteria thriving in a normal human gut are not just a stagnant force; they outnumber the body's own cells and aid in the digestion of foods, the protection of the body, the secretion of vitamins and hormones, and the basic function of the intestinal tract. Therefore, when these bacteria change due to a modified diet high in fats, they can become harmful to the body and cause inflammation. For example, the bacteria eventually escape the intestine and they can elicit chronic systemic inflammation in different areas of the body, with the help of lipopolysaccharides (LPS), and cause a multitude of inflammatory diseases, such as Type 2 Diabetes, Alzheimer's Disease, Crohn's Disease, and Rheumatoid Arthritis.

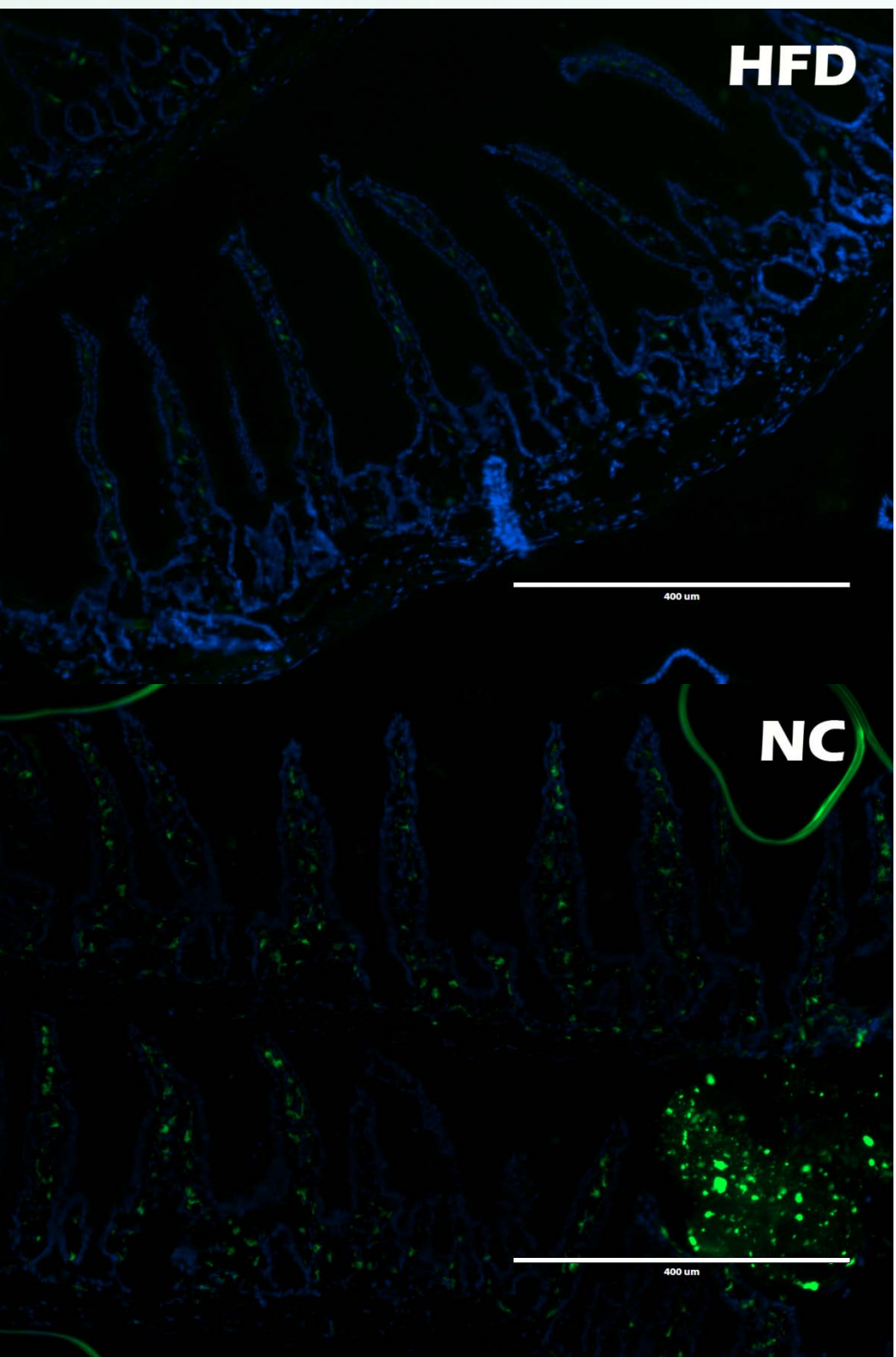


In our last three weeks of research, we aimed to better understand how the bacteria escapes the gut. We have been trying to make a stronger correlation between the diet and the inflammation that may occur in the intestine as a result of the diet, so that we can understand the reasoning behind what is causing these inflammatory diseases. To test this hypothesis, we looked at the differences between slices of intestines from mice raised on a normal diet and a high fat diet. We checked for the concentration of macrophages and eosinophils in the distal small intestine and the colon, since these two types of white blood cells are both major proponents of the body's immune response to fighting off foreign pathogens. Macrophages are phagocytic cells involved in the innate immune response, which is a non-specialized defense against all types of pathogens. Eosinophils have multiple purposes but their primary job is to aid the macrophages in protecting the body. To determine the concentrations of eosinophils and macrophages, we stained the slices of intestine we made from high fat diet and normal chow mice with various fluorescent dyes to be able to view them underneath a microscope. We then counted the number of macrophages and eosinophils in each villus and compiled our data to make conclusions about what ultimately might be the cause of inflammatory diseases.

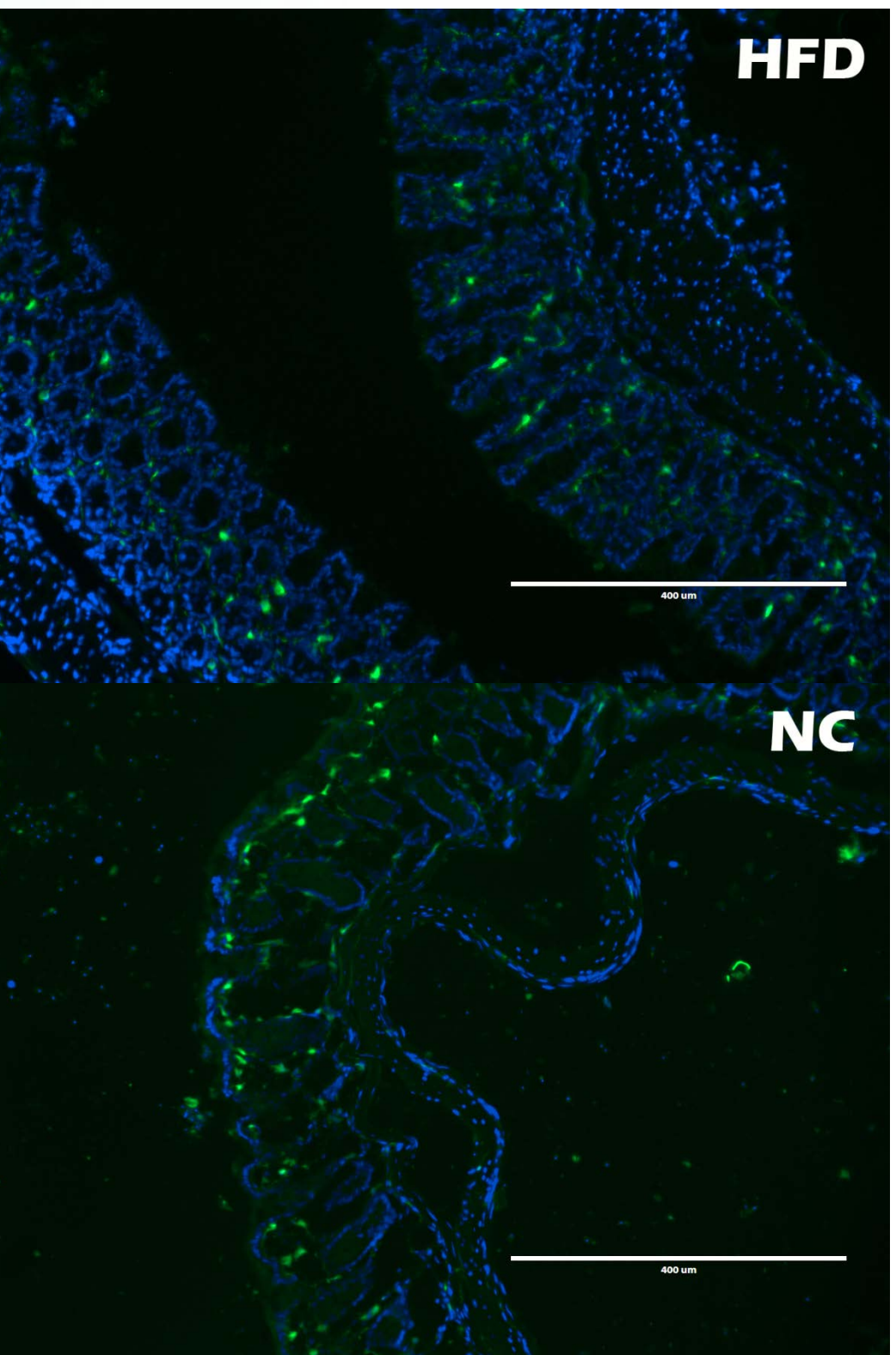
Macrophage Concentrations in the Guts of Various Mice

DAPI and GFP Staining in the Intestines at 10x Magnification

Small Intestine



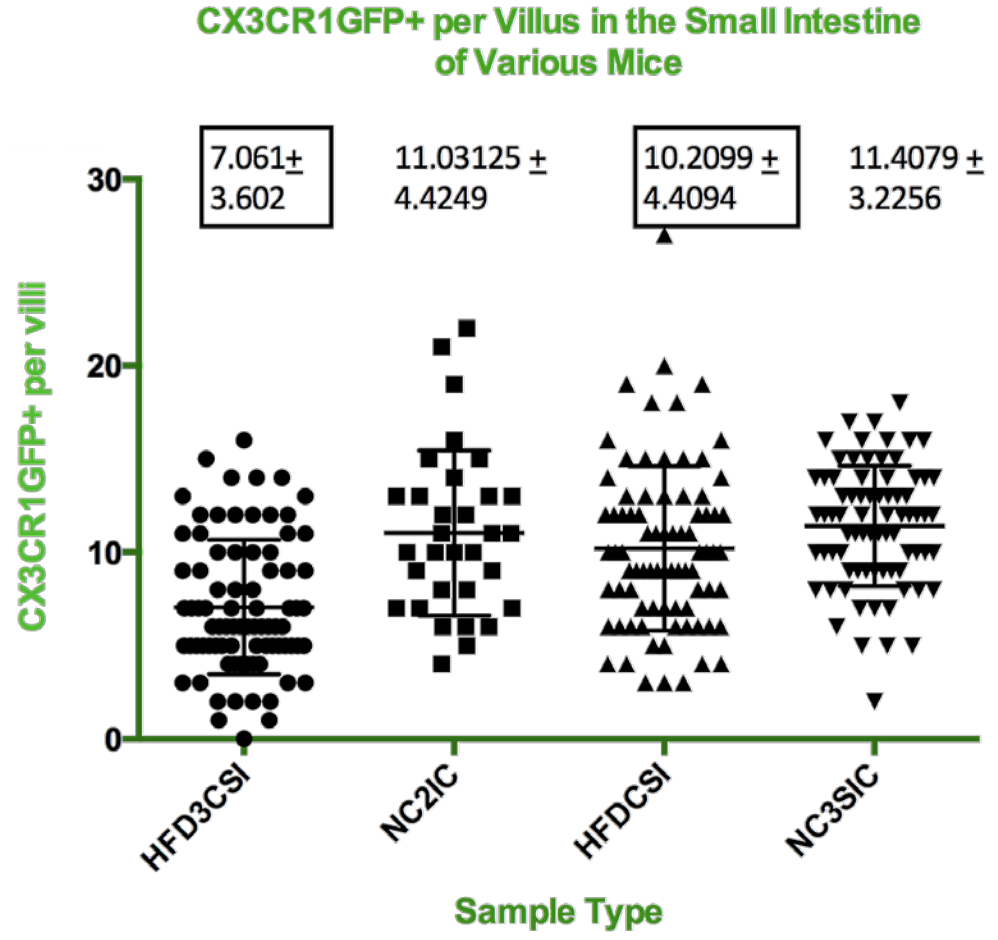
Colon



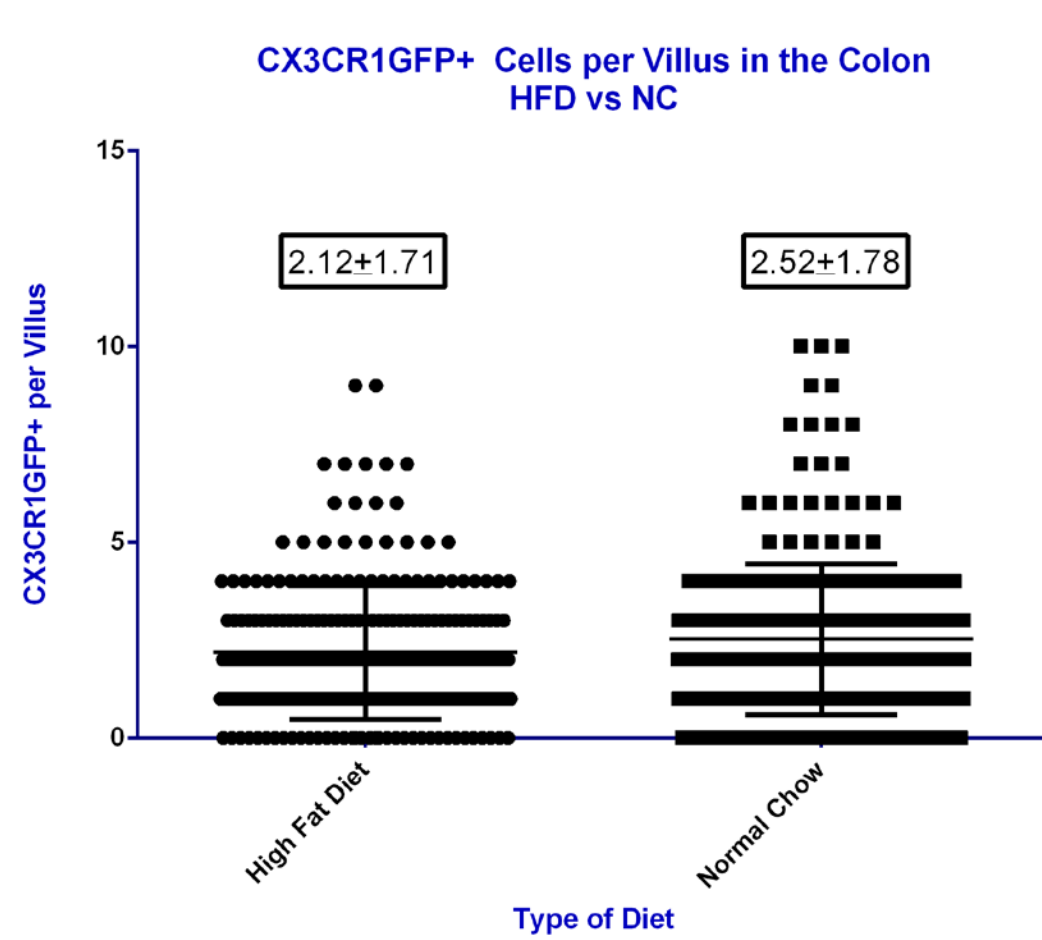
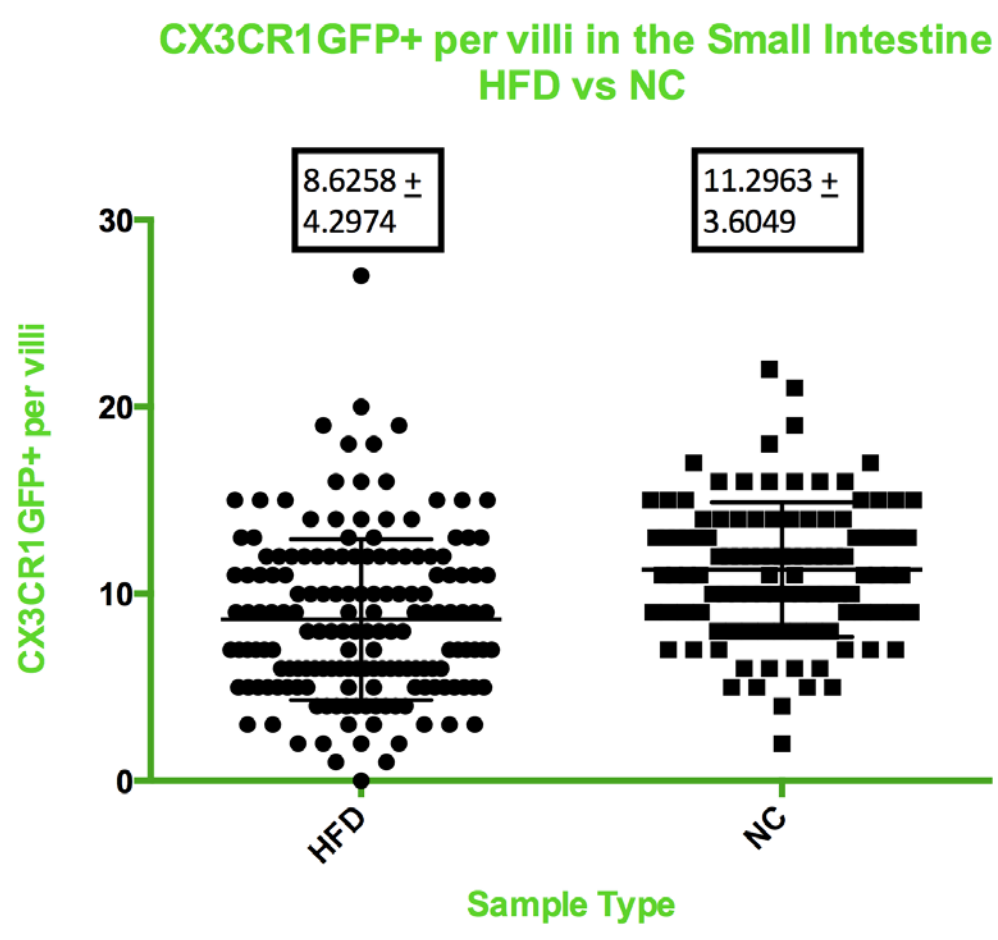
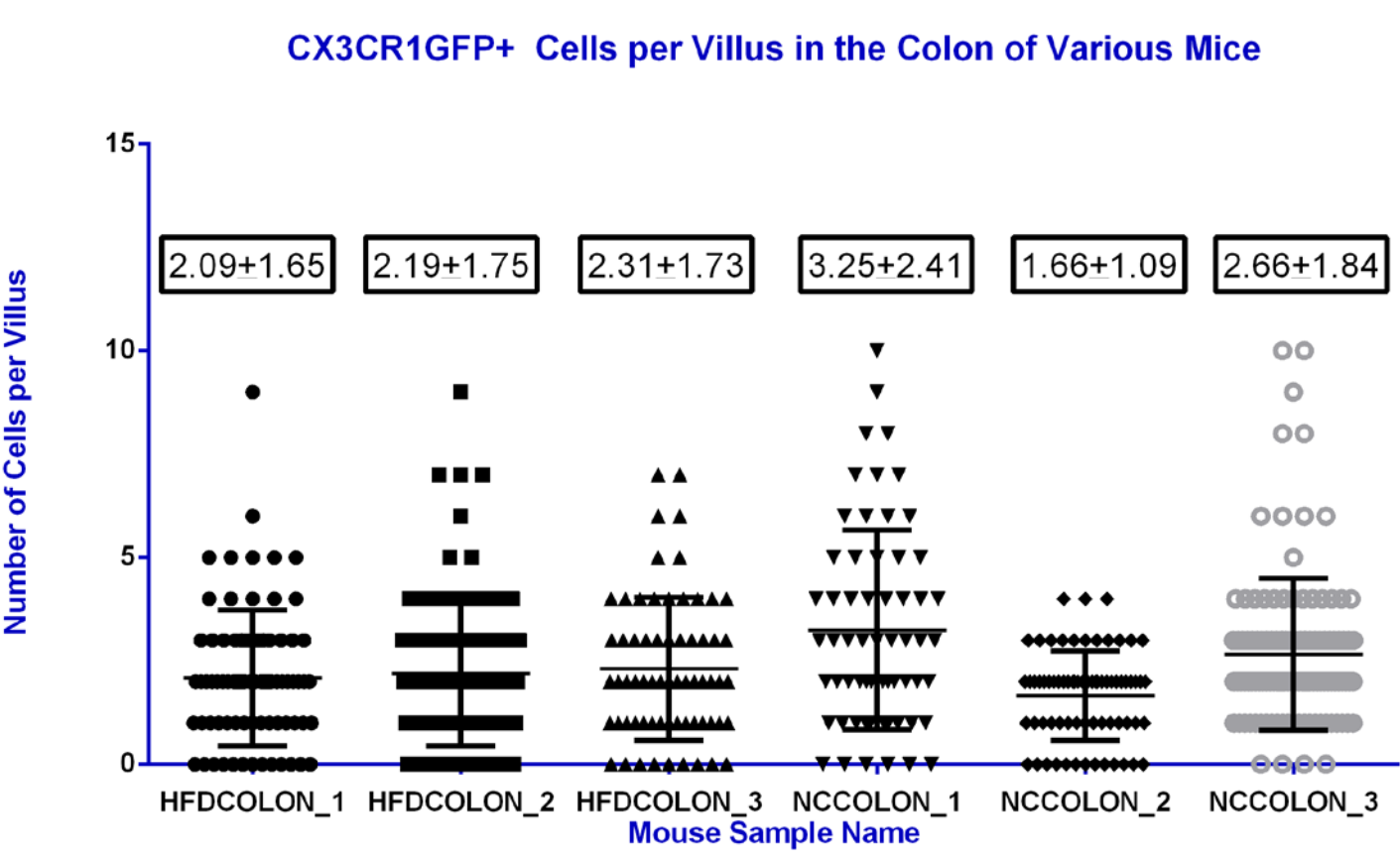
The pictures above are sections of the small intestine and colon from different mice fed with either a high-fat diet (HFD) or normal chow (NC). Each section is cut seven microns thick using a cryostat and stained with GFP and DAPI. The GFP highlights CX3CR1GFP+ cells in a green color. CX3CR1GFP+ cells are also known as macrophages. Macrophages are white blood cells involved in the innate immune response. The DAPI highlights the nuclei in all cells in a blue color.

CX3CR1 GFP+ Cells Per Villus

Small Intestine



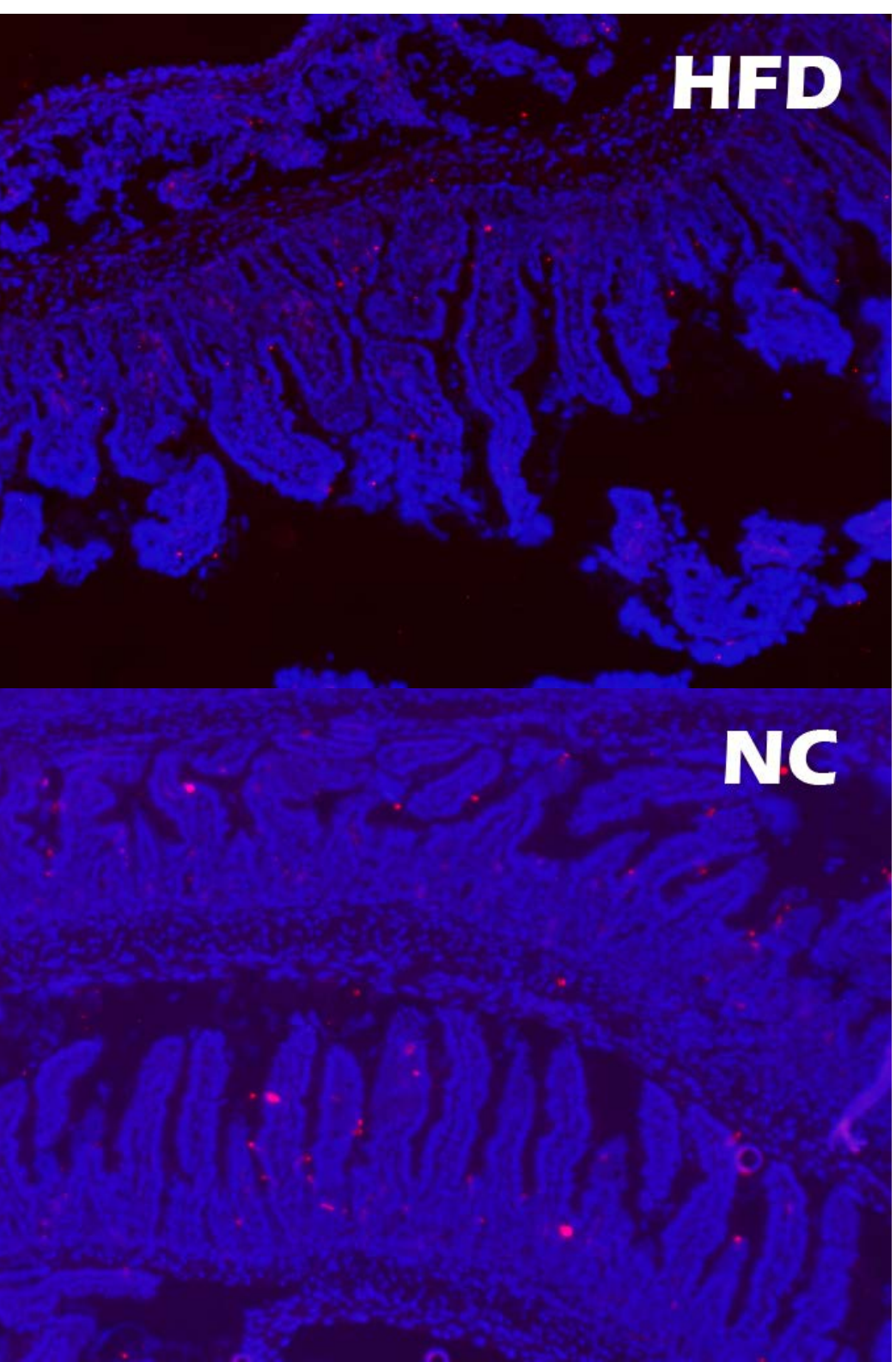
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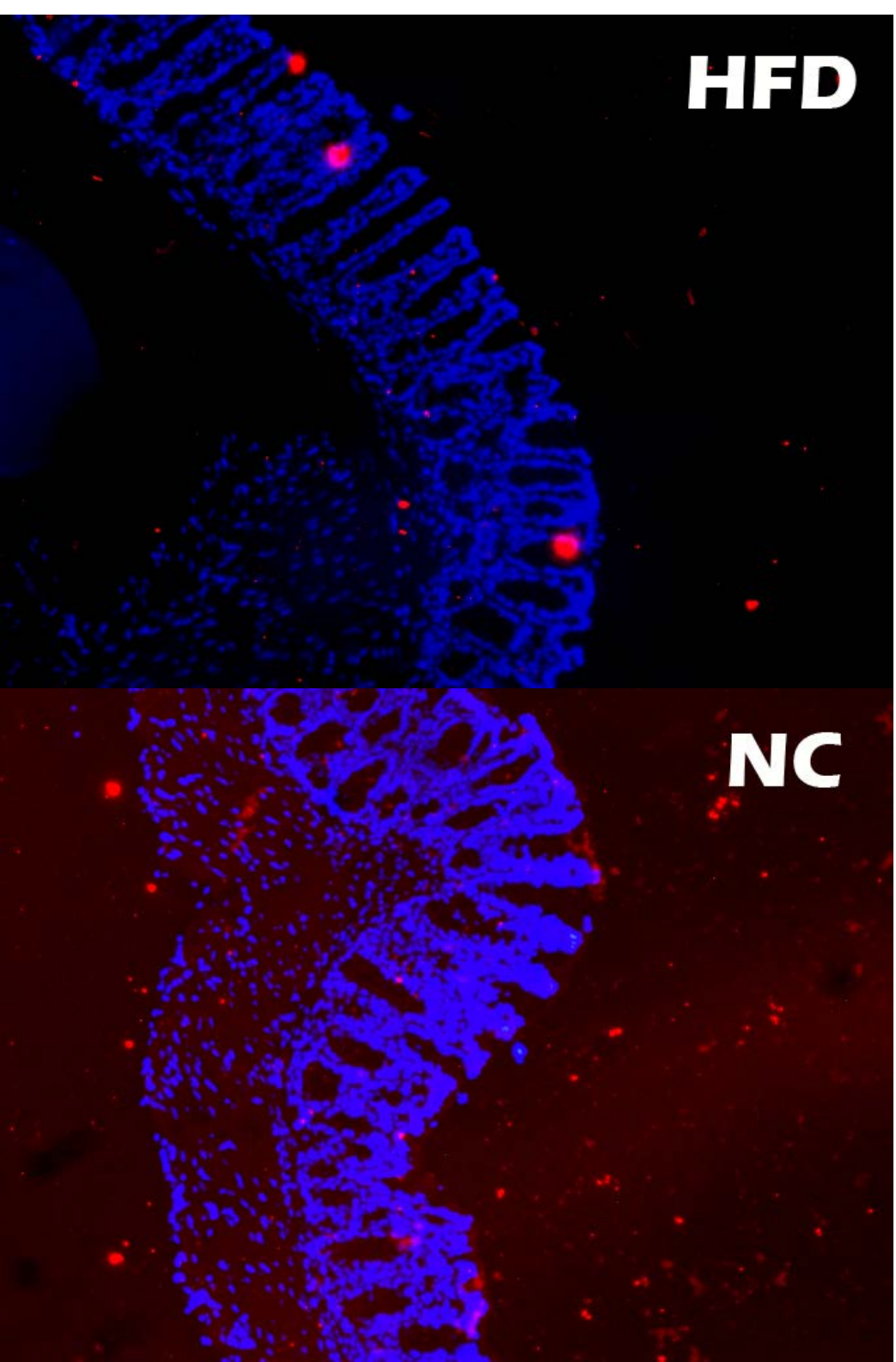
These graphs depict the number of macrophages that we counted in each villus from the distal small intestine and colon of high fat diet (HFD) and normal chow (NC) mice. Each point on the graph represents the number of macrophages that we identified in one villus of one type of mouse. A general trend throughout all of the graphs is that normal chow mice tend to have more macrophages per villus than high-fat diet mice do, in the small intestine as well as the colon. This data could mean that mice fed with normal food are more inflamed than mice fed with fatty foods, or that there is a defect with the immune system in high-fat diet mice.

Eosinophil Concentrations in the Guts of Various Mice

Small Intestine

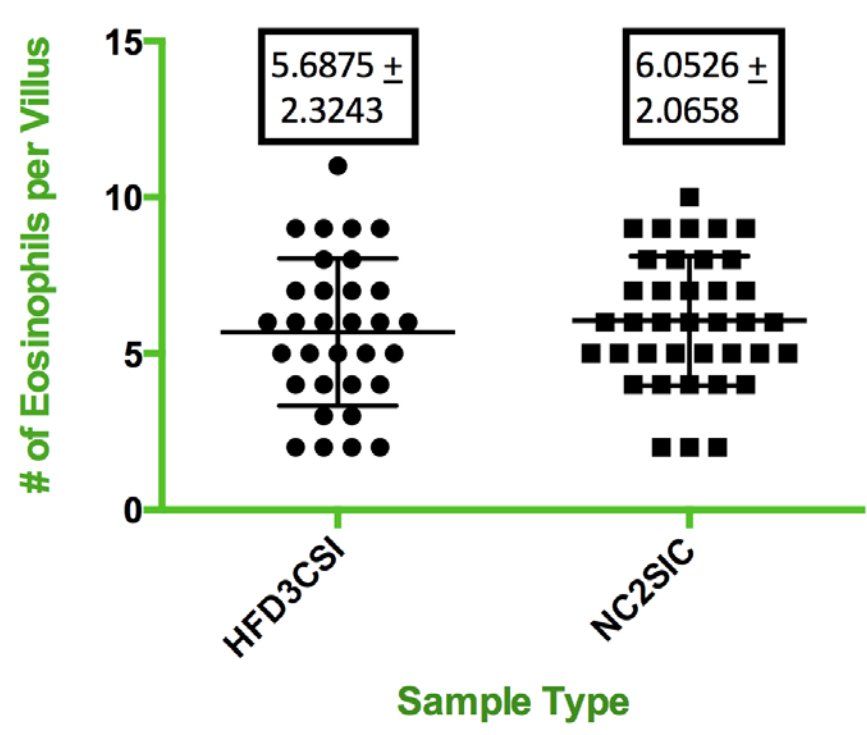


Colon

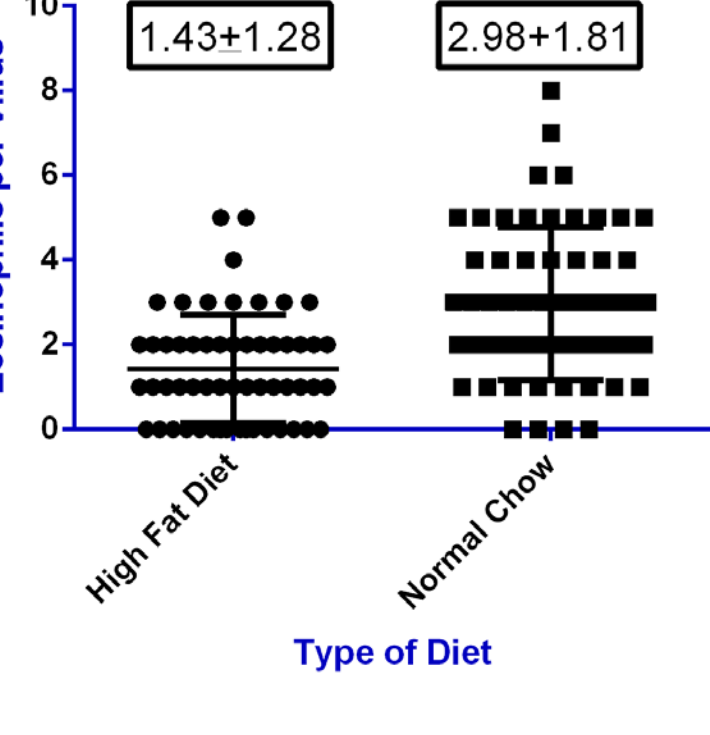


The sections above are also sections of the small intestine and colon that are cut seven microns thick on the cryostat. These sections were stained with DAPI to identify each cell nucleus and with a mix of anti-SIGLEC-F antibodies and PE dye to identify the eosinophils. When added to the slides, the anti-SIGLEC-F antibodies stained with PE bind to the antigen receptors on the eosinophils, so that each eosinophil could be identified by the fluorescent antibody staining. This experiment is important because eosinophils, along with macrophages, are white blood cells that are involved in the immune response.

Eosinophil per Villus in the Small Intestine HFD vs NC



Eosinophil Count per Villus in the Colon HFD vs NC



CONCLUSION

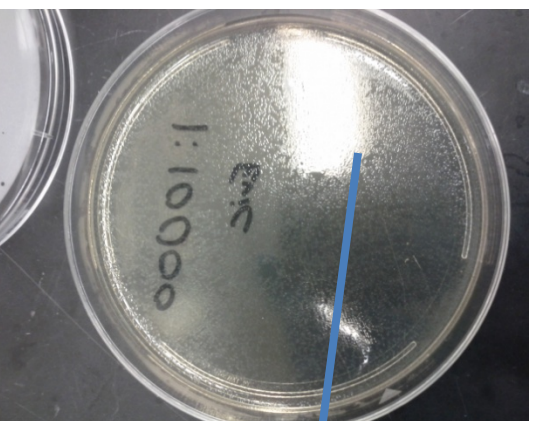
The data that we generated regarding macrophage and eosinophil counts in both the distal small intestine and colon show a reduction in immune activity in mice fed with a high-fat diet compared to the ones fed with normal chow. Unlike our initial perception that the gut would be more inflamed in high-fat diet mice due to the inflammation in the rest of the body, there were fewer macrophages in high-fat diet mice than in normal chow mice. These results show that there is a diminished immune response in mice fed with fatty foods, which could also explain how bacteria from the gut are able to escape the intestine and infect other parts of the body without being detected, causing chronic systemic inflammation. The gut microflora that is corrupted by the fat and cholesterol could escape detection from the macrophages and eosinophils because the immune system's cells could be engulfing the extra fat causing them to be unable to function properly.

FUTURE EXPERIMENTS

- ❖ Further look into eosinophil counts with a confocal microscope
- ❖ Infect mice on HFD with bacteria to confirm a lack of immune response
- ❖ Analyze macrophage/eosinophil cell deaths using apoptosis assays (Annexin V or Tunnel)

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Magnified Image